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MINERAL DEFICIENCIES IN DERRIS ELLIPTICA

by
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Plant Physiologist

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By RUFUS H. MOORE, *plant physiologist* [1]

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INTRODUCTION

Derris elliptica (Wall.) Benth, is a legume indigenous to islands of the southwest Pacific and the neighboring Asiatic mainland. A forest liana, it responds somewhat unfavorably when grown out of its natural environment. In the open field in Puerto Rico, *Derris* has exhibited few and mild abnormal responses, even though the broad leaflets on the pinnately compound leaf of this dicotyledonous plant should favor the development of specific symptoms (*24*).[2] Occasionally leaves are slightly bleached by direct rays of the midday sun even in the rainy season. The combination of drought, direct sunlight, and wind may desiccate the tips of leaflets. The color change in new leaves exposed to the sun is modified by available moisture: When the water supply is adequate the red pigment of young leaves disappears as chlorophyll develops, but drought causes some new leaves to change from red to yellow and then slowly to green. In addition, the potential chlorophyll-bearing tissue bordering the distal part of principal veins is sometimes especially slow to turn green, giving such leaves a "white-vein" appearance. Tiny brown spots that develop on some leaflets formed late in the dry season do not appear on new leaves formed after spring rains have induced a growth flush (fig. 1). The

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[2] Italic numbers in parentheses refer to Literature Cited, p. 24.

basal part of the blade may develop chlorophyll faster than the distal part. Occasionally new leaves fall off when still very small and unopened. The possibility that some of these symptoms might be the result of nutrient deficiency suggested the experiment described herein.

FIGURE 1.—Leaf of the Sarawak Creeping variety of *Derris elliptica* showing the necrotic brown spots that appear during the dry season.

Many reports have appeared on the responses of plants to mineral deficiencies, ranging from observation of general symptoms to exhaustive research on histological and chemical changes induced by the omission of essential elements. Cation and anion triangles are a useful departure in the technique for studying deficiencies (*15*, *44*). Inasmuch as *Derris* is relatively new as a cultivated crop, investigations on the behavior of this species under mineral-deficiency treatment have

been few (*22*, *29*). This report gives the details of an experiment (*30*, *pp. 10–11*) on the symptomology of certain nutrient deficiencies in *Derris* and shows the relationship between these deficiencies and the chemical composition of the roots.

PROCEDURE

Selection of Plants

Relatively large plants with thin roots had to be used because (1) rotenone does not accumulate in an appreciable concentration until roots are 2 mm. or more in diameter (*28*, *31*); (2) among roots of the same diameter rotenone content may vary more than 100 percent (*28*); (3) once stored in roots, rotenone remains unchanged by treatment that reduces carbohydrates to extremely low levels in the derris plant (*28*); and (4) a comparatively large sample of roots is needed for estimating rotenone and total extractives. Thin roots were desirable in order to minimize the error introduced by the amount of rotenone in them at the beginning of the test period; but to be sure that the deficiency plants would develop roots of appreciable thickness before the experiment was terminated, it was necessary to use plants with a considerable food reserve. Such reserves were doubtless accompanied by greater absolute amounts of deficiency elements than would have existed in smaller plants.

A clone of the Changi No. 3 variety was prepared as follows: Cuttings, 10 to 15 mm. in diameter, were kept in a field nursery until well rooted. Just before removing them from the nursery, the tops of the plants were cut back to short stumps. Only plants with thin roots were transferred to white sand and supplied with tap water to encourage the proliferation of fine roots. After 3 weeks selected plants were washed free of sand and transplanted individually to 5-gallon glazed coffee-urn liners filled with coarse quartz sand practically free of plant nutrients.[3]

Filtered tap water was applied for 3 weeks to allow the plants to renew growth before the mineral-deficient solutions were started on August 18, 1941.

Experimental Conditions

The plants were grown in a greenhouse with a glass roof and hardware-cloth sides. Urns, supported on wooden frames that allowed free drainage, were spaced 2 feet apart along the edges of a low greenhouse bench 4½ feet wide. The results of deficiencies of sulfur, nitrogen, phosphorus, calcium, potassium, iron, and magnesium were determined by comparing plants under these treatments with those given a complete nutrient solution. The four plants in each of the eight treatments were randomized so that each of them constituted a replicate. The vines were trained on galvanized wire and confined to their respective parts of the trellis (figs. 2 and 3).

The water used to prepare the nutrient solutions was secured from a 10-gallon tin-coil still. Nutrients entrained from the boiler were

[3] The sand contained 0.05 percent of iron oxides, 0.004 percent of lime, and 0.015 percent of magnesia.

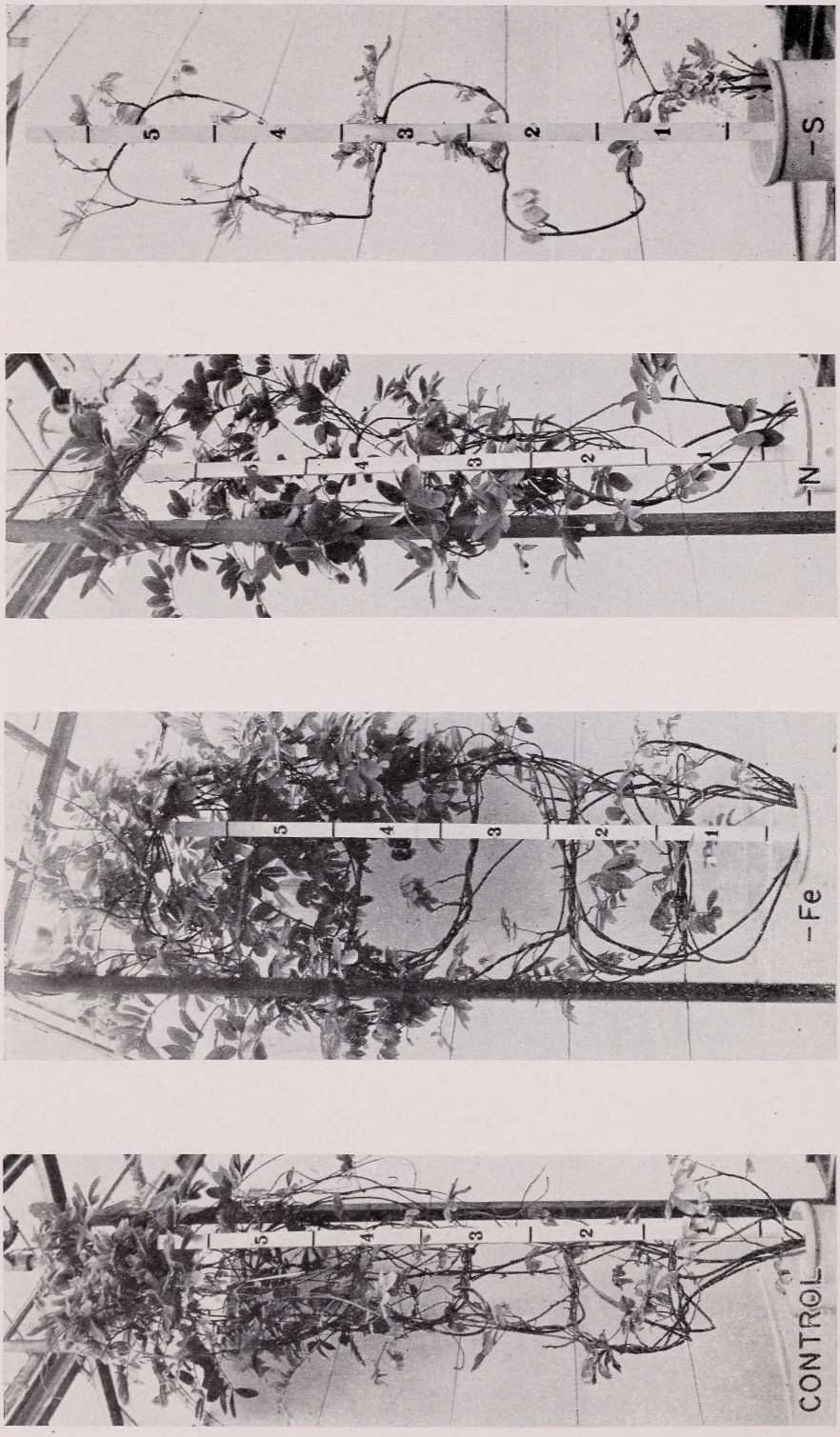

FIGURE 2.—Derris plants of the control, —Fe, —N, and —S treatments just prior to harvest. Lines on measuring stick indicate feet.

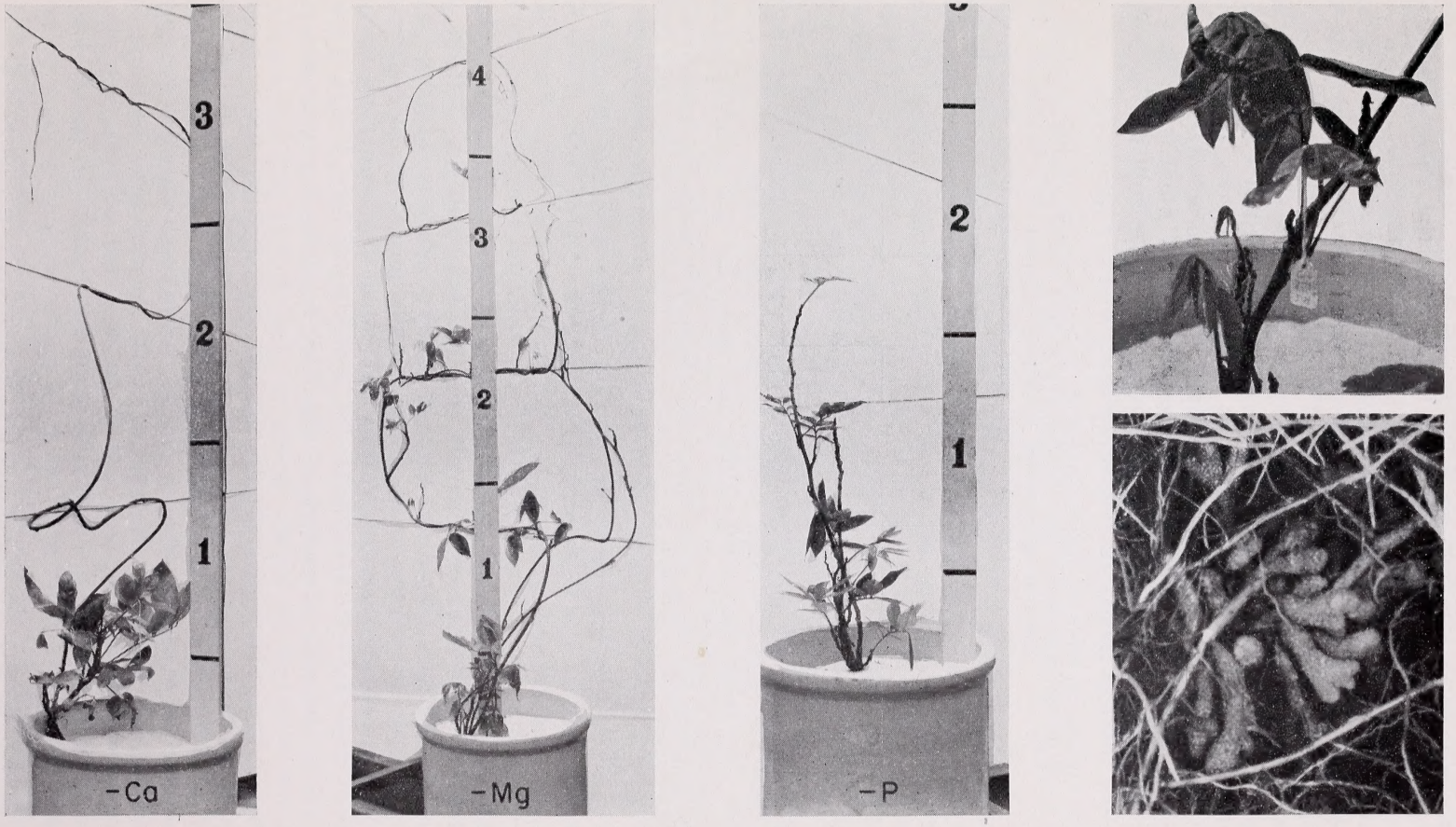

FIGURE 3.—Derris plants of the —Ca, —Mg, and —P treatments just prior to harvest. Calcium deficiency in leaf in upper right-hand corner. Dendriform nodules on roots of a —N plant are illustrated in lower right-hand corner, ×0.44.

almost completely removed by washing the steam through an 8-inch layer of water on the bottom of the still. As used, the water contained a trace of calcium and from 1 to 2 p. p. m. of magnesium.

Nutrient solutions were modifications of those recommended by Hoagland and Arnon (*17*) and were prepared in carboys air-brushed on the outside first with black enamel and then with aluminum paint. The volume of solution applied daily to each culture depended on the size of the plant and varied from ½ liter to more than 4 liters. Progressive increases in transpiration as the more vigorous plants grew made it necessary to dilute all nutrient solutions to one-half strength after 3 months and to one-third strength after 7 months. Table 1 gives the concentrations of mineral nutrients in the solutions as used from the seventh month to the termination of the experiment.

TABLE 1.—*Milliequivalents per liter of ions in nutrient solutions used in studying mineral deficiencies of Derris elliptica*

Ion	Nutrient solutions [1]						
	Control and −Fe	−N	−K	−P	−Ca	−Mg	−S
	M. e.	*M. e.*	*M. e.*	*M. e.*	*M. e.*	*M. e.*	*M. e.*
NH_4	0.25						
NO_3	4.92		3.33	4.67	1.67	4.67	5.67
K	2.33	1.67		2.00	2.33	3.33	2.00
H_2PO_4	.33	.33	.33		.33	.33	.33
Ca	2.67	1.66	5.00	4.00		4.00	2.67
Mg	1.33	1.33	1.33	1.33	1.33		1.33
SO_4	1.33	4.33	2.67	2.66	1.66	2.33	

[1] Boron, manganese, zinc, copper, and molybdenum as recommended by Hoagland and Arnon (*17*) were added to all nutrient solutions. Equivalents of concentrated solutions of ferric chloride and sodium citrate were mixed and diluted to supply iron at 1 to 2 p. p. m.

To help stabilize the pH of the solutions in the sand, the ammonium ion was added at the beginning of the test as NH_4NO_3 to the −S, −Fe, and control solutions and as $(NH_4)_2SO_4$ to the −P, −Ca, −K, and −Mg solutions. When the pH of the solution that dripped from the sand was lowered to 5.0, ammonium was reduced or omitted. After 3 months only the −Fe and control solutions required the addition of this ion. Acidity in the sand solution of the −N series became accentuated, probably because of the accumulation of residual sulfate ions. To prevent values lower than pH 4.0 in the sand solution of these cultures, NaOH was added to the nutrient solution; but care was taken to avoid adding enough of this base to cause a precipitate to form.

When cultures were periodically flushed with distilled water, fresh nutrient solutions were added immediately. The pH value of the first 50 ml. of solution to drip from the jars was determined at intervals, and the NH_4NO_3, $(NH_4)_2SO_4$, or NaOH in the fresh nutrient solution was adjusted to maintain the hydrogen-ion concentration of the drip as closely as possible between pH 5 and 6. The pH values of the nutrient solutions, estimated by a Taylor color comparator, are summarized in table 2.

TABLE 2.—*The pH values of nutrient solutions as applied to and as recovered from sand cultures. Data for recovered nutrient solutions include average deviations*

Nutrient solution	Control	−Fe	−N	−K	−P	−Ca	−Mg	−S
	pH	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*
Applied	5.1	5.0	5.6	4.9	5.1	5.1	5.1	5.0
Recovered	6.0±0.6	5.7±0.8	4.7±0.9	5.4±0.5	5.7±0.5	5.8±0.6	5.5±0.6	5.7±0.4

Records for each plant were also kept on the cycle of leaf and stem flushes, on abscission, size, color pattern and malformation of leaves, and on stem dieback.

Leaf Tests

When foliar symptoms of deficiency had become pronounced, the ion withheld from a nutrient solution was applied to leaflets (*28*). Numerous hairs prevented the penetration of water to the stomata, which are confined to the lower epidermis. To obtain penetration of the test solution to the stomata it was necessary to rub the leaflets gently between the thumb and index finger wet with distilled water until a film clung to the lower epidermis. A thin strip of absorbent cotton ¼ inch wide was wrapped around the wet part of the leaflet and held in place by a folded strip of celluloid fastened with a paper clip. The test solution was then added from a small pipette until the cotton was saturated. Cut 1½ inches wide, the celluloid reduced evaporation from the cotton. Leaflets that had just unfolded were most suitable for this test. In all cases except that of −S, the chloroses to be described were not obvious or their distinctive patterns had not fully developed on these young leaflets. Tests usually extended over the period from 9 a. m. to 3 p. m., so that the solutions would be in contact with the epidermis during the period when stomatal opening was normally at its maximum.

Harvest

The experiment was terminated 12 months after the application of deficiency solutions was begun. At that time the lengths of living and of dead stems were recorded and the tops were discarded.

Sand was dislodged from the roots by a jet of tap water. One or more pieces ¼ inch long were cut from the thickest roots of each plant and air-dried for microchemical tests. The rest of each root system was sorted into the following diameter groups: 0–1.9 mm., 2.0–4.9, 5.0–9.9, and 10 mm. or more. None of the roots was more than 15 mm. in diameter. Fresh weights were taken, and the volume of roots in each diameter group was determined by water displacement. Roots 2 mm. or more in diameter from each plant were put into one sample and cut into strips 1 mm. or less in thickness. All roots were heated in an aerated electric oven at 80° C. for 30 to 45 minutes and then transferred to a 60° oven for 1 to 1½ hours to reduce their moisture content to an approximately air-dry condition. The small amount of sand which stuck to fresh fine roots loosened during the drying process and was removed. Samples were stored in double manila paper bags until ground for analysis.

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Chemical Methods

Drying.—All samples were ground through the 60-mesh sieve of an F. R. I.-model Wiley mill and dried 20 hours at 55° C. in a vacuum oven at 100 mm. Hg. Drying reduced the moisture from an average of 10.5 to 2.5 percent. The samples were stored over calcium chloride until analyzed.

Red-color value.[4]—Since many of the 32 samples were so small that the Official method of estimating rotenone could not be employed, the red-color value, representing rotenone plus rotenoids, was determined colorimetrically in a Coleman spectrophotometer (*20*).

Total extractives and rotenone.[4]—The four individual samples of small or large roots in each series were composited in proportion to their respective weights and analyzed gravimetrically for total chloroform extractives (*12*) and rotenone (*2*).

Total nitrogen plus nitrates.—From 10 to 20 mg. of the composite samples was used to determine total nitrogen plus nitrates by the micro-Kjeldahl adaptation of the reduced iron method (*27*).

Protein nitrogen.—The difference between total nitrogen plus nitrates and water-soluble nitrogen is reported as protein nitrogen.

Soluble nitrogen fractions.—Water extracts were prepared from 0.3 to 0.5 gram of the composite samples by a modification of the method of Vickery et al. (*43*). The powder was made into a thin paste with a small quantity of distilled water, diluted to about 85 ml., heated 10 minutes with constant stirring in a water bath at 80° C. to precipitate water-soluble proteins, allowed to cool, diluted to 100 ml., shaken thoroughly several times during the course of 30 minutes, transferred to oil tubes, and centrifuged 5 minutes. As centrifuging for 20 minutes at 2,500 r. p. m. did not clarify some of the preparations perceptibly more than centrifuging for 5 minutes at a lower speed, it was necessary to employ other means to secure clear solutions.

Since material not removed by the first centrifuging was roughly proportional to the red-color values of the samples, chloroform was tried as a clarifying agent. The liquids of the first centrifuging were decanted into another set of dry oil tubes, 5 ml. of choloroform was added to each, the tubes were shaken vigorously, and the samples were centrifuged 10 minutes. The chloroform completed the clarification. In decanting the clarified solutions, the chloroform frequently loosened from the constricted base of the oil tube and clouded the extract again. To avoid such resuspension of the precipitate, all but approximately 1 ml. of the chloroform was pipetted from the bottom of the oil tubes and the samples were centrifuged again for 2 minutes. The completely clarified solutions could then be decanted without the intermediate precaution of passing them through dry funnels plugged with glass wool. The fact that no precipitate formed during the acid hydrolysis of the amide determination was evidence that clarification was complete. The extracts were protected with toluene and kept in a refrigerator. Analyses were completed within a few days after the dry samples had been extracted.

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[4] Chemical analyses for red-color value, total extractives, and rotenone were made by M. A. Jones, chemist, Federal Experiment Station in Puerto Rico, Mayaguez.

Nitrate nitrogen.—This was determined by the Official phenoldisulfonic acid method (*2*), with minor modifications. The yellowish color of the clarified solutions was removed by dispersing 50 mg. of decolorizing charcoal in 10-ml. aliquots in 50-ml. Erlenmeyer flasks, removing air from the charcoal by momentary suction, stoppering the flasks and swirling 2 minutes in a water bath at 50° C., allowing them to cool 30 minutes, and filtering the extract through dry filter paper. Since only faint traces of chlorides were present, their precipitation by Ag_2SO_4 solution was omitted. Five-ml. portions of the decolorized extracts were dried on a steam bath, completely dissolved within 2 minutes in 2 ml. of phenoldisulfonic acid reagent, diluted with 50 ml. of water, made alkaline with an excess of concentrated NH_4OH, transferred to 100-ml. volumetric flasks, cooled to room temperature, made to volume, and thoroughly mixed. Nitrate nitrogen was estimated colorimetrically in a Coleman spectrophotometer at wave length 410 mμ with a slit width of 30 mμ and using distilled water as the reference.

Ammonium and amide nitrogen.—These were estimated on separate 10-ml. aliquots of the clarified solution (*42*). Water-soluble nitrogen was determined on 1-ml. aliquots of the clarified solution by the micro-Kjeldahl adaptation of the reduced-iron method (*27*).

Soluble organic nitrogen.—This fraction is expressed as the difference between water-soluble nitrogen and the sum of ammonium and nitrate nitrogen.

RESULTS AND DISCUSSION

Gross Symptoms of Deficiencies

None of the variations from normal appearance of derris leaves, reported in the Introduction to occur on field-grown plants, was correlated with specific mineral deficiencies in the greenhouse. Tipburn, which appeared in all treatments, was usually preceded by loss of turgor in the part of the leaflet that eventually died, and became more pronounced when sun, heat, and wind affected the plants adversely. "White-vein" was mild in most cases and was frequently associated with tipburn. Tiny necrotic brown spots, which appeared on the leaflets of plants under all treatments except −Ca and −P, were a mild symptom from January through April. The tendency of a few young leaflets to develop chlorophyll in their basal half before it appeared in the distal half was observed in all treatments except −S. Twisting, cupping, or other distortion of tips of young leaflets occurred in −Ca, −Mg, −S, −Fe, and control plants: These symptoms were most pronounced in −Ca, and least in control plants. The occasional abscission of unopened new leaves was common to all treatments.

In addition to the foregoing nonspecific variations from normal that have been observed on both field and greenhouse plants, another such variation developed on the greenhouse plants only. Superficial black streaks appeared on the petioles and midribs of older leaves in all treatments except −Ca. This superficial blackening became most pronounced on leaves of the −S plants, probably because leaf abscission was notably delayed by sulfur deficiency.

Differences in anthocyanin content of young derris leaves deserve comment. Several months after deficiency treatments had been in

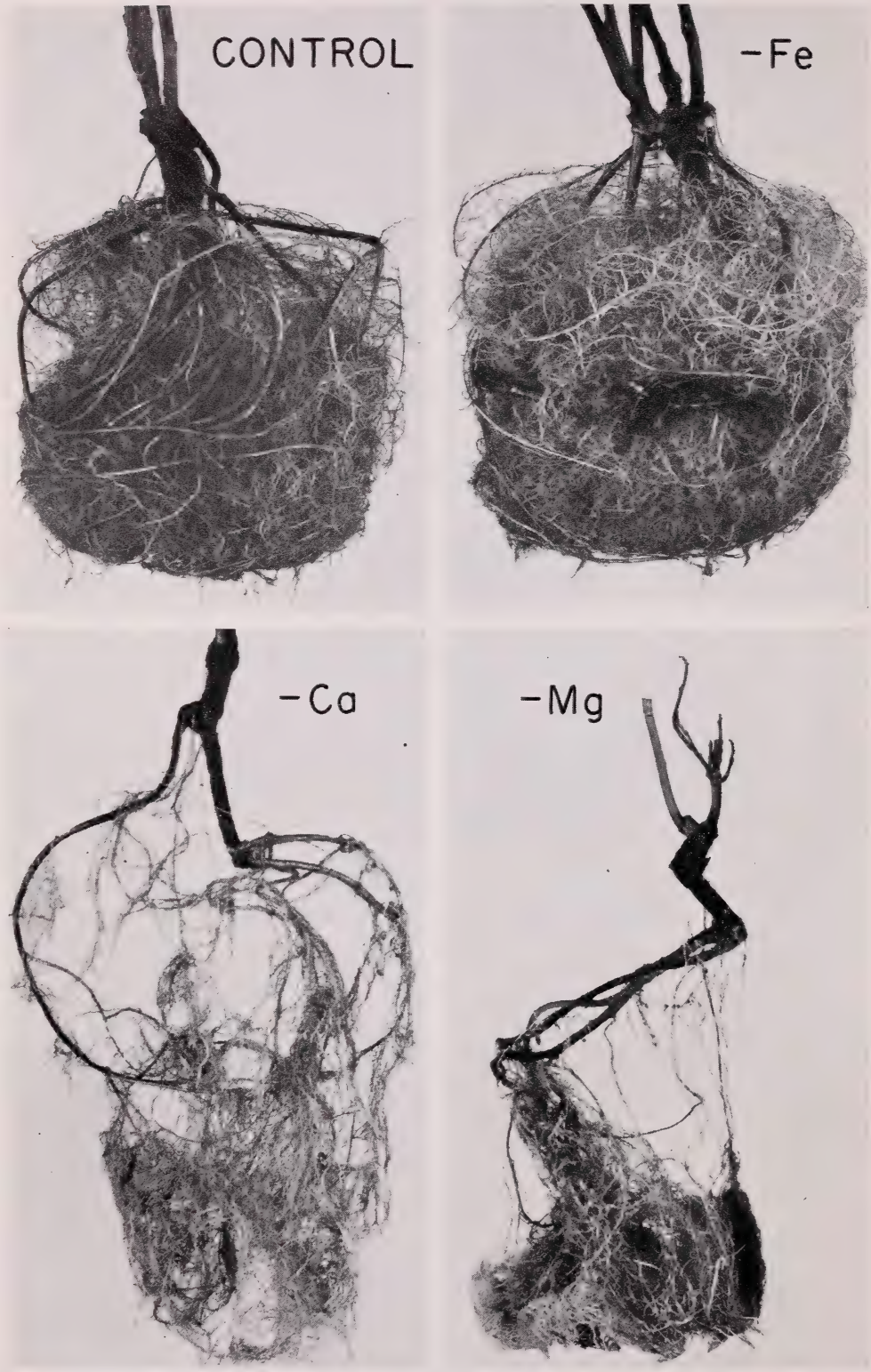

FIGURE 4.—Root systems of derris plants from each of the 8 treatments. Photographic reduction, ×0.18, is the same throughout.

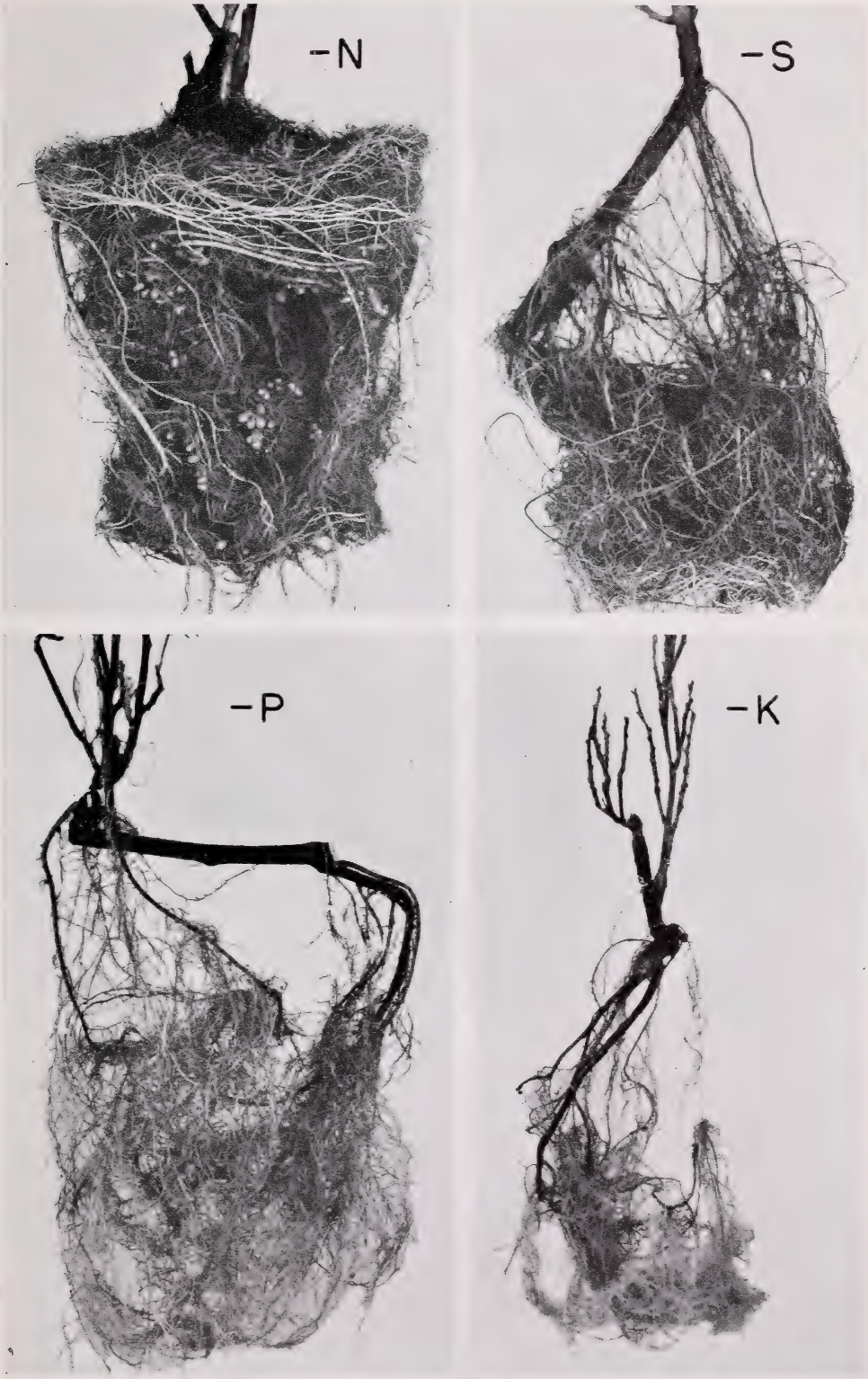

FIGURE 4.—Continued.

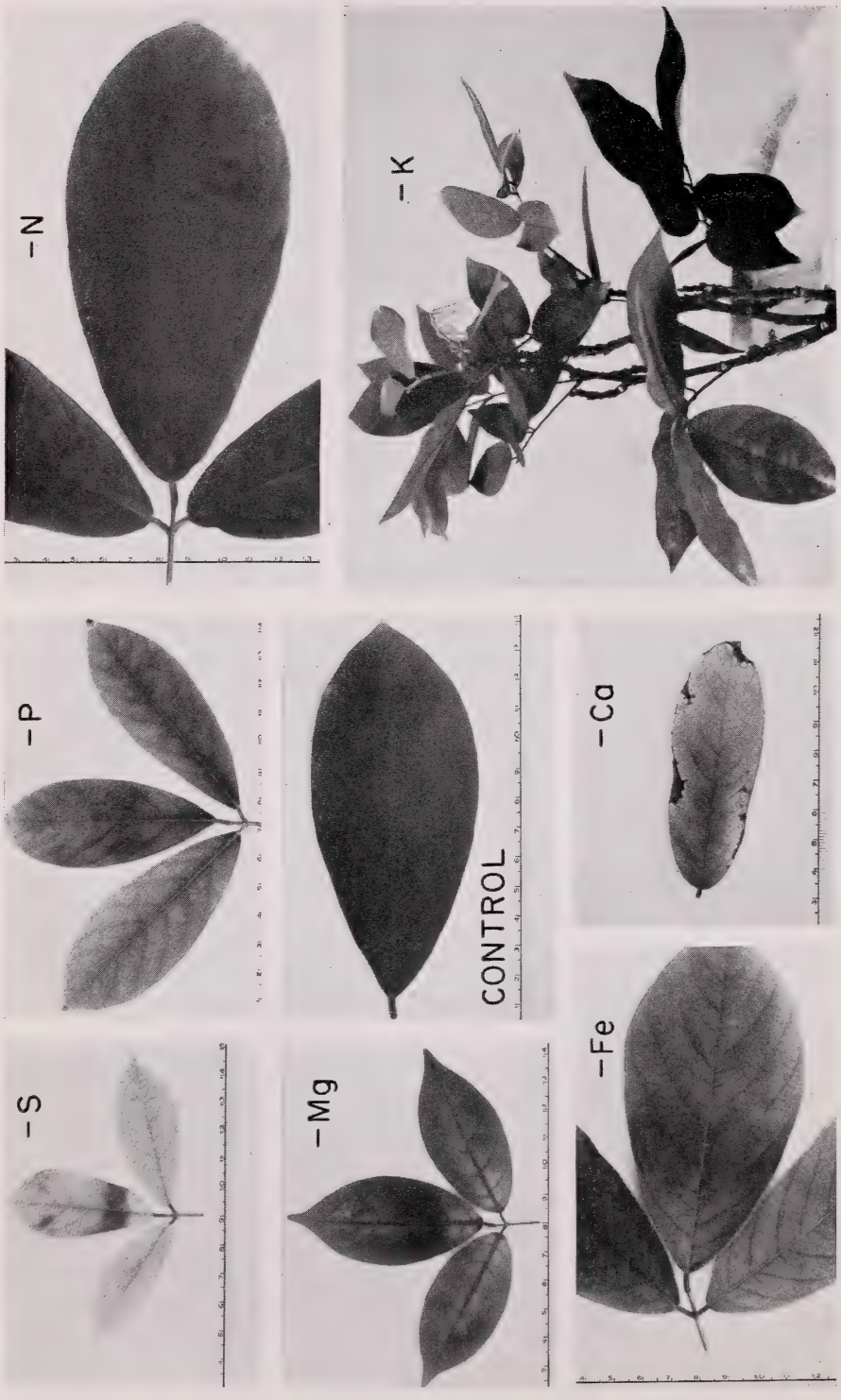

FIGURE 5.—Top row: Leaflets of —S, —P, and —N plants. Middle row: Leaflets of —Mg and control plants. Bottom row: Leaflets of —Fe and —Ca plants and a —K plant. The center leaflets of —S, —P, —Mg, and —Fe groups show bands of tissue which turned green when the respective deficiency ions were applied.

progress, the pink or reddish pigment that normally precedes chlorophyll was more intense in −N and −Mg leaves and absent from −K and −S leaves. It did not appear in −P and −Ca cultures except in the leaves on slow-growing shoot flushes late in the experiment.

Some degree of stunting (table 3) was the only symptom observed on *Derris* which is common to other species of plants growing in mineral-deficient media (*25*). The more specific symptoms of mineral deficiencies in *Derris* described hereafter are illustrated in figures 2, 3, 4, and 5.

TABLE 3.—*Stem length and dry matter of roots of mineral-deficient derris plants as compared with controls*

Treatment	All stems		Living stems		Mean dry matter of roots	
	Total length	Control	Total length	Part of all stems in treatment	Per plant	Control
	Meters	*Percent*	*Meters*	*Percent*	*Grams*	*Percent*
Control	72.2	100	67.9	94	90.3	100
−S	8.9	12	8.0	90	29.1	32
−N	29.3	41	27.3	93	[1] 81.5	90
−P	3.3	5	2.6	81	13.4	15
−Ca	5.4	8	.8	15	9.5	11
−K	1.3	2	1.2	95	6.4	7
−Fe	64.4	89	60.4	94	73.5	81
−Mg	11.3	16	5.1	46	10.8	12

[1] Not including nodules: Healthy, 13.9 grams; dead, 1.9 grams.

Minus sulfur.—Shoot flushes in −S plants were fewer and more protracted than in plants under any other treatment. Within 2½ months a few thick vines began to grow near the base of old stems. Elongating with remarkable rapidity, they reached 8 to 10 feet in length before their growth rate became imperceptible. Although the production of vines was reduced by sulfur deficiency, the percentage that remained alive compared favorably with that of the controls (table 3).

Yellowing of leaves and stems characterizes sulfur deficiency (*9*, *36*). The decline in pigmentation of derris leaves became noticeable in less than 3 months and appeared as a blotching of several shades of green to green yellow. Leaves formed later were yellow along the borders of veins. As the plants continued to grow, new leaves became a uniform pale greenish yellow and finally a clear yellow. The gradual reduction in green pigments was accompanied by a corresponding decrease in leaf size. Eight months were required for −S plants to produce new leaves and stems that were completely yellow. Abscission was greatly retarded in old leaves, which slowly developed an irregular pattern of pale green, became desiccated at the tips, and developed considerable blackening of petioles and principal veins before they fell off.

Older fine roots were grayish to brownish and similar in diameter to those of control plants. Bright-colored new roots were thickened, from 2 to 4 inches long, and grew from the older fine roots by an abrupt increase in diameter.

Minus nitrogen.—The progressive yellowing and extreme stunting of the shoot induced by lack of nitrogen in the nutrient solution of other species of plants (*14*, *22*, *33*) did not appear in *Derris* owing to

the fact that no attempt was made to keep the derris cultures free of nodular bacteria. Vigorous new shoots developed near the base of old stems. Although the total length of vines on −N plants was only two-fifths of that of controls, it was from 1½ to 19½ times as much as that of vines in the other deficiency series except −Fe. Mature leaves were not noticeably reduced in size and were at best only slightly less green than those of control plants.

With 28 percent more dry matter than the controls, the roots of −N *Derris* tended to be like those of other species grown without nitrogen. Withholding nitrogen from the nutrient solution favored the growth of exceptionally large nodules that were frequently dendriform and that provided considerable nitrogen to the plants (table 3, figs. 3 and 4).

Minus phosphorus.—Stunting was so pronounced that −P plants produced only 5 percent as much total stem length as controls. New leaves were not greatly reduced in size until late in the course of the experiment. The first foliar symptom specific to sulfur deficiency appeared on old leaves 3 months after treatments were started and consisted of a bluish-green cast in the chlorophyll-bearing tissue adjoining the midrib and principal veins. New leaves continued to be uniformly pale green for some time after they had completed growth. Gradually the diffuse bluish cast would appear along the midrib and veins, the other leaf tissues meanwhile becoming slightly paler. The abscission of old leaves was noticeably delayed. Healthy fine roots were pale in color, stiffish, definitely thicker, and less frequently branched than roots of control plants.

Minus calcium.—The −Ca plants, with only 8 percent as much vine length as controls, ranked third in degree of stunting due to mineral deficiencies. The greatest mortality of vines occurred in this treatment, 85 percent of all vines dying before harvest. After 9 months of deficiency treatment, most of the original vines had died and new shoots began to grow from the top of the old cuttings buried in the sand. Within 2 months the single new shoots on each of two plants had grown to 20 and 36 inches in length.

Although several foliar symptoms appeared on one or more plants, none of them was consistent enough to be considered specific for calcium deficiency. Newly opened leaves were pale green. The entire network of veins often became noticeably greener, and the islets of mesophyll lost some of their green so that the leaflets developed a chlorotic pattern resembling the initial stage of iron deficiency. Leaves were moderately to greatly reduced in size.

Infrequently, small irregular water-soaked spots near the up-curled tips and margins of young leaflets caused considerable distortion (fig. 3). Tipburn of very young leaves was more common in −Ca plants than in those of other deficiencies. Leaflets not affected by tipburn when young sometimes showed this symptom later as a narrow, irregular band that included much of the leaflet margin (fig. 5). The characteristically acuminate tips were apparently lost from a few leaflets on one leaf when very young, resulting in rounded tips resembling those on calcium-deficient tobacco plants (*14*, *24*).

The newest roots of the two plants that had developed shoots shortly before harvest were whitish, but the newest roots of the other two plants were darker. Older fine roots of all plants were much-

branched, medium to dark gray, and often apparently dead. No roots had noticeably bulbous tips.

Minus potassium.—The plants in this series were so stunted that they produced only 2 percent as much stem growth as the controls. The foreshortening of internodes was extreme (fig. 4). Leaves were soon considerably reduced in size and frequently fell off while still green. The color of the leaves was generally an intense blue green (fig. 5). The development of anthocyanins, found to accompany potassium deficiency in other species (*14*, *35*), was observed on only two of the four −K derris plants. In both cases the purpling of midrib and principal veins was observed on the leaflets of single leaves exposed to direct sunlight. Even though tipburn appeared, −K plants (*14*) were less affected by it than plants under other treatments. The newest fine roots were weak and much smaller in diameter than those on plants in other series.

Minus iron.—The checking of stem growth by iron deficiency was scarcely significant. The first mild foliar symptoms of iron deficiency appeared 4½ months after the −Fe solution was first applied, and not all plants showed them until nearly 9 months had elapsed. New leaves were pale green and soon developed a more intense green in the border parenchyma of the entire network of veins, leaving the islets of mesophyll pale by contrast. At no time did foliar symptoms approach the severity that has occasionally been observed in the field or that was induced in high-carbohydrate plants in sand cultures forced into rapid growth by a nutrient solution high in nitrogen (*28*). Roots were healthy and medium brown and formed a dense mat in the sand.

The low concentrations of phosphate and manganese (*41*) in the nutrient solution, the reserve of iron in the plants themselves, and possibly also the iron oxide visible in the sand were factors that contributed to the mild symptoms in plants under this treatment.

Minus magnesium.—Although the extent of reduction of stem length caused by the lack of magnesium was practically the same as that induced by sulfur deficiency, the rate of stem elongation of −Mg plants was slow. During the first 6 months of the experiment, the foreshortening of internodes was not pronounced, but during the last 6 months scarcely any stem elongation was observed. More than half of the stems died during the course of the experiment.

New leaves were notably reduced in size by the end of 5 months and fell off shortly after they had expanded. The rhythm of leaf flushes began to disappear at the end of 6 months; and from the ninth to the twelfth month the plants produced a continuous succession of leaves that, on the average, were progressively smaller as the experiment was brought to a close. At the end of 5 months some of the maturing leaflets on all plants began to show pale strips between the principal veins—the first evidence considered to be typical of magnesium deficiency. The chlorotic pattern became more definite on leaves formed subsequently. Destruction of chlorophyll, which began near the midrib, progressed outward and eventually almost reached the leaflet margin, sometimes involving even the chlorenchyma bordering the principal veins. Before affected leaves fell off, the blanched tissues frequently became dry and brown. Similar symptoms have been illustrated for tomato (*14*, *p. 185*). In a few leaves the complete network

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of veins remained green while the intervening tissues turned almost white, giving a pattern like that which characterizes magnesium deficiency in tobacco (*24*). This symptom did not appear on all plants.

Roots suffered severely. Fine roots were weak and light to medium grayish brown, and many were dead. Some roots as thick as 5 mm. had become so soft that they had to be discarded. Only roots firm enough to retain their shape for determination of their volume by water displacement were saved for chemical analysis.

Supplying Deficiency Ions to Leaflets

Ions withheld from plants were first applied to leaflets at three times their respective concentrations in the complete nutrient solution of table 1. When this concentration was ineffective or only partly satisfactory, the strength of the solution was doubled repeatedly until chlorosis was corrected or the leaflet tissues were injured. In no case was a solution used stronger than 48 times its tabulated concentration. Results were positive only on leaflets that might develop a chlorotic pattern which would become intensified with time. The response to treatment was confined to the leaf tissue in direct contact with the saturated cotton band (fig. 5).

A solution of Na_2SO_4 with 4 m. e. of the sulfate ion per liter was effective on a few small spots of the treated tissue. The concentration had to be raised to 16 m. e. in order to secure a uniform color change. Within 2 days the treated part of the leaflet turned pink with anthocyanins, and during the next 3 days the anthocyanins disappeared as the green pigments of chlorophyll developed.

Nitrogen was applied as NH_4NO_3. Even a concentration of 248 m. e. of nitrogen was completely ineffective in hastening the development of chlorophyll in young pale-green leaflets of $-N$ plants.

It was necessary to use a solution of NaH_2PO_4 at a concentration of 8 m. e. to secure a satisfactory response in $-P$ leaflets. The development of maximum green in affected tissues required 2 weeks.

Even though the leaflets of $-Ca$ plants were always paler than normal and frequently developed chlorotic patterns, the application of a solution of $CaCl_2$ did not modify these symptoms on older leaves or prevent their development on younger leaves. The test solution with 128 m. e. of calcium injured immature leaf tissue.

Since no chlorotic pattern characterized K-deficient leaflets, supplying potassium ions in a KCl solution caused no color change. A concentration of 112 m. e. of potassium induced no increase in the size of leaflets.

A solution of ferric citrate applied at 10 p. p. m. of Fe induced chlorophyll formation in the mildly chlorotic islets between fine veins within 4 days after treatment, and the green became gradually more intense over a period of 1 week. Ferric citrate was not so satisfactory as the ferrous or ferric sulfate used in another experiment (*28*).

The foliar symptoms considered typical of Mg deficiency were corrected by 4 m. e. of $MgCl_2$. Greening of what would have been chlorotic tissue was complete in 5 to 14 days. Had the distilled water for nutrient solutions been entirely free of magnesium, the pattern of chlorosis might have been different and a higher concentration of magnesium ions required to prevent chlorosis in treated leaflets.

General Discussion of Foliar Symptoms

Although foliar symptoms of a mineral deficiency are frequently similar in different species, their variations are too great to use any one species as a standard for diagnosis (*14*). On the whole, the foliar symptoms induced by mineral deficiencies in *Derris elliptica* resemble those described for one or more of several other species, the most notable exception being the lack of chlorotic or necrotic symptoms characteristic of −K plants.

In all probability the foliar symptoms attributed to mineral deficiencies resulted specifically from the withholding of ions from the nutrient solutions. The fact that characteristic chlorotic patterns either did not appear or did not continue to develop in young leaves to which missing ions were added was direct evidence that such ions were needed by the leaf and indirect evidence that the entire plant was also deficient in them.

Leaf tests were inconclusive on plants lacking nitrogen, potassium, or calcium in their nutrient solutions. In the case of −N plants this result was associated with the rapid development of chlorophyll, the normal green having developed in such a short time that any effect of added ions was completely masked. The application of KCl solution to leaflets of −K plants produced no apparent change because there was no potential chlorotic pattern to be prevented. Lacking the evidence of leaf analysis, it cannot be stated with certainty whether the intense blue green and abscission of leaves of −K plants might or might not have been caused by the accumulation of the ammonium ion (*45*).

The failure of species to develop consistent deficiency symptoms, such as those already described for *Derris elliptica* in −Ca cultures, has been encountered in several species of plants under other mineral-deficiency treatments (*14*). The death of stem tips, distortion and necrosis of leaflet margins and tips (*24*, *34*), and the varied patterns of chlorosis of −Ca derris plants were doubtless caused by insufficient calcium. Stem, leaf, and root symptoms were not consistent, probably because both the distilled water used for nutrient solutions and the sand in which the plants were grown had a trace of calcium.

Unfavorable conditions, known to induce pathological symptoms which could be misinterpreted (*1*, *5*, *11*, *18*, *26*, *34*, *41*, *45*), were avoided so far as possible. For example, Fe deficiency, accentuated by a relative excess of soluble manganese or phosphate, was slow to appear in −Fe *Derris*, and symptoms never became severe. It should be stated, however, that responses peculiar to *Derris* were not exhaustively studied in this exploratory test.

Microchemical Tests on Roots

Thin, freehand sections of the air-dry root pieces were tested for nitrate, starch, and rotenone and rotenoids, enough water being added to wet the sections before reagents were applied.

The diphenylamine sulfate test for nitrates was strongly positive in the roots of all plants save those in the −N and −K series. Roots of −N plants were completely negative, and those of −K plants were moderately positive.

The starch-iodide test was strongly positive in the −N and control roots, positive in −P and −Fe, moderately positive in −S and −K, fair to poor in −Ca, and almost negative in −Mg. Starch remained in cells surrounding vessels when it had practically disappeared from other starch-storing tissues.

The Durham test for rotenone and chemically similar compounds involves the use of HNO_3 (1+1) followed by an excess of concentrated NH_4OH (*19*). Although originally intended for tissue extracts, this test can be made directly on thin sections of plant organs. Applied to root sections of plants grown in full sunlight, the HNO_3 caused an intensely red color to develop in most of the rotenoid cells. When the surplus acid was drawn off with a pipette and the sections flooded with an excess of NH_4OH, the red compounds turned blue or bluish green momentarily. In a few seconds the rotenoid cells darkened, many of them becoming black.

Mineral deficiencies as studied under greenhouse conditions induced variations in the number, distribution, and staining capacity of rotenoid cells. Differences in number and distribution were most striking in −S and −Mg roots. The rotenoid cells in −S roots became progressively more numerous as new tissues were added to both phloem and xylem; but, by contrast, few and scattered rotenoid cells were differentiated in the corresponding tissues of −Mg roots. The pattern of rotenoid cells in roots of the other treatments fell between these two extremes. Differences in staining capacity were clearly illustrated in the −N and −Fe series. Although both had an abundance of rotenoid cells, the staining reaction was more intense in the −N than in the −Fe roots. In −Ca roots the number of rotenoid cells appeared to be fewer but their staining reaction more intense than the average for all treatments.

Since quality varies more than 100 percent among roots, even of the same diameter, the root pieces saved for microchemical studies were too few to make a completely satisfactory study of the comparative number, distribution, and staining properties of rotenoid cells. The microscopic observations do, however, shed light on the histological basis for the differences in quality revealed by colorimetric and gravimetric analyses.

Root Quality

Worsley (*47*) found that deficiencies of nitrogen, calcium, potassium, and phosphorus increased the percentage of rotenone in dried derris roots. As his test was admittedly of a preliminary nature, many of the details essential to interpretation of results were not reported.

The effect of a mineral deficiency on the concentration of rotenone or of rotenone plus rotenoids in derris roots is more precisely expressed on a fresh-volumetric basis than on an absolute, dry-matter, or fresh-weight basis. The absolute basis does not take into account the essential criterion of concentration because of large differences in the amounts of dry matter in roots of the several treatments. For example, the control plants, with an average yield of 90.3 grams of dry matter and 2.44 grams of rotenone plus rotenoids, would rank higher in quality on an absolute basis than the −S plants for which the respective yields were 29.1 and 1.57 grams. When data were

expressed on any basis other than the absolute, control plants were definitely inferior to −S plants. Table 4 shows that dry-matter data would alter some of the conclusions that could be drawn; and fresh-weight data do not eliminate the small error introduced by the specific gravity of the starch and other substances that were replaced by water in certain treatments.

TABLE 4.—*Means of dry matter and of rotonone plus rotenoids in roots of control and mineral-deficient derris plants*

Treatment	Roots less than 2 mm. in diameter			Roots 2 mm. or more in diameter					All roots		
	Dry matter	Rotenone plus rotenoids		Dry matter	Rotenone plus rotenoids		Calculated mean diameter of fresh roots	Volumetric part of all roots	Dry matter	Rotenone plus rotenoids	
		Dry-matter basis	Fresh-volumetric basis		Dry-matter basis	Fresh-volumetric basis				Dry-matter basis	Fresh-volumetric basis
	Pct.	*Pct.**	*Mg./cm.³**	*Pct.*	*Pct.**	*Mg./cm.³**	*Mm.*	*Pct.*	*Pct.*	*Pct.*	*Mg./cm.³*
Control	12.2	1.4±0.2	1.7±0.2	32.4	3.9±1.2	13.8±3.5	5.1	30	18.7	2.7	5.1
−S	11.2	3.0± .5	3.4± .6	26.3	7.2± .9	20.4±3.2	5.6	34	16.6	5.4	9.2
−N	14.7	2.0± .2	3.0± .3	40.7	3.9± .2	17.9±1.5	5.5	21	20.5	2.9	6.2
−P	10.7	2.0± .3	2.3± .6	29.5	5.3±1.0	16.5±2.7	6.5	23	15.3	3.5	5.8
−Ca	9.5	2.5± .3	2.4± .1	25.7	4.9±1.5	13.5±2.7	6.0	31	14.7	3.9	5.9
−K	10.1	1.7± .5	1.8± .4	26.2	5.5±1.6	15.7±3.4	5.0	20	13.5	3.3	4.7
−Fe	11.6	1.4± .1	1.6± .1	27.4	3.9± .4	11.6±2.3	5.9	30	16.5	2.7	4.6
−Mg	5.9	1.0± .3	.6± .2	19.9	5.4±1.2	11.2±2.3	4.6	18	8.7	2.9	2.6
Odds 19 to 1 [1]		0.38	0.79		0.46	5.15					2.14
Odds 99 to 1 [1]		.51	1.07		.63	7.01					2.91

*'The average deviation follows each value.'
[1] Differences necessary for significance at odds indicated.

Table 5 gives the statistical significance of the differences in milligrams per cubic centimeter of rotenone plus rotenoids among the small and the large roots of the several treatments. Considering all roots of each plant as a unit, statistical analysis of the volumetric data in table 4 showed that the concentration of rotenone plus rotenoids in the the roots of the −S plants was greater by high significance than in roots under any other treatment. The −S roots, with 9.2 mg. per cubic centimeter, had 48 percent more rotenone plus rotenoids than −N roots, 80 percent more than control roots, and 254 percent more than −Mg roots. Also, the −N, −P, and −Ca roots were superior by high significance to −Mg roots in content of rotenone plus rotenoids; but the control roots were only significantly superior. The −S and −Mg roots were the only ones that showed any statistical significance in relation to the control.

TABLE 5.—*Statistical differences in milligrams per cubic centimeter of rotenone plus rotenoids in derris roots*

Odds	Small roots	Large roots	All roots
99 to 1	−S> −Mg, −Fe, C, −K, −P. −N> −Mg, −Fe, C. −Ca, −P, −K> −Mg.	−S> −Mg, −Fe.	−S> −Mg, −Fe, −K, C, −P, −Ca, −N. −N, −Ca, −P> −Mg.
19 to 1	−S> −Ca. −N> −K. −K, C, −Fe> −Mg.	−S> C, −Ca. −N> −Mg, −Fe. −P> −Mg.	C> −Mg.

Gravimetric analyses of composite samples are given in table 6. On a dry-matter basis, roots of the control plants in this greenhouse experiment had only 1.3 percent of rotenone in contrast to 5.2 percent in trellised plants of the same clone grown out-of-doors (*31*). A large part of the difference in these rotenone values can be attributed to the high proportion of fine roots on the greenhouse plants grown in sand in comparison to the low proportion of fine roots on the outdoor plants grown in clay.

TABLE 6.—*Percentages of total chloroform extractives and rotenone in dry composite root samples of control and mineral-deficient derris plants*

Treatment	Total chloroform extractives in—			Rotenone in—		
	Small roots	Large roots	All roots	Small roots	Large roots	All roots
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Control	2.0	4.8	3.6	0.7	1.8	1.3
−S	5.2	9.2	7.4	1.2	3.4	2.4
−N	3.1	4.8	3.9	.9	1.4	1.1
−P	3.8	6.6	5.1	.4	2.0	1.1
−Ca	4.0	7.1	5.7	.2	1.8	1.1
−K	2.8	7.8	4.8	(1)	.8	
−Fe	2.2	5.1	3.7	.6	1.8	1.2
−Mg	1.8	7.2	4.2	1.0	1.9	1.4

[1] Sample too small for rotenone analysis.

Relation Between Root Quality and Soluble Organic Nitrogen

In general, plants grown at a high nitrogen level of nutrition have a relatively large percentage of their organic nitrogen in water-soluble forms (*33*): *Derris* grown at a high nitrogen level stores more rotenone than at a low nitrogen level (*32*). On the other hand, the concentration of soluble organic nitrogen increases in plants from which sulfur (*9*, *36*), calcium under special conditions (*34*), phosphorus (*10*, *23*), and potassium (*46*) have been withheld: derris plants in the −S, −Ca, and −P treatments were higher in rotenone plus rotenoids than the controls. These facts made it seem probable that a positive correlation between the rotenone-plus-rotenoid and soluble organic nitrogen values of derris roots might be established.

That such a correlation existed is evident in the data of tables 7 and 8 and in figure 6. The −S roots had the highest concentrations of both rotenone plus rotenoids and of soluble organic nitrogen, and the −Mg roots had the lowest concentrations of these constituents. Figure 6 shows a more consistent correlation between rotenone-plus-rotenoid and soluble organic nitrogen values in small than in large roots. Inasmuch as most of the tissues of small roots were formed while deficiency treatments were in progress, the results for small roots are a more reliable index to the effects of treatments than those for large roots. Notable deviations from correlation occurred in the large −Ca and control roots. With the exception of these two cases, most of the discrepancy in correlation can be attributed to the small number of plants in each treatment.

TABLE 7.—*Percentages of nitrogen fractions in dry roots of control and mineral-deficient derris plants*

Treatment	Total nitrogen plus nitrates		Protein nitrogen		Soluble organic nitrogen		Amide nitrogen		Ammonium nitrogen		Nitrate nitrogen	
	Small roots	Large roots	Small roots	Large roots	Small roots	Large roots	Small roots	Large roots	Small roots	Large roots	Small roots	Large roots
	Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
Control	2.946	2.260	2.370	0.267	0.119	1.752	0.049	0.042	0.052	0.063	0.405	0.178
−S	3.310	3.647	1.368	.795	1.397	2.393	.019	.466	.158	.109	.387	.350
−N	2.888	1.941	2.283	.807	.531	1.082	.041	.349	.074	.052	.000	.000
−P	3.044	3.183	1.794	.933	.487	1.905	.061	.506	.043	.051	.720	.294
−Ca	2.726	1.685	.934	.834	1.286	.297	.072	.059	.078	.051	.428	.563
−K	2.970	2.402	2.329	.891	.372	1.325	.085	.399	.130	.095	.139	.091
−Fe	3.363	2.688	2.684	.769	.242	.993	.057	.303	.044	.072	.393	.254
−Mg	3.116	1.660	2.805	.880	.107	.148	.003	.056	.065	.086	.139	.546

TABLE 8.—*Milligrams per cubic centimeter of some nitrogen fractions in fresh roots of control and mineral-deficient derris plants, and percentages of soluble organic nitrogen in total nitrogen plus nitrates*

Treatment	Total nitrogen plus nitrates		Protein nitrogen		Soluble organic nitrogen		Soluble organic nitrogen in total nitrogen plus nitrates	
	Small roots	Large roots	Small roots	Large roots	Small roots	Large roots	Small roots	Large roots
	Mg./cm.^{3}	*Mg./cm.*^{3}	*Mg./cm.*^{3}	*Mg./cm.*^{3}	*Mg./cm.*^{3}	*Mg./cm.*^{3}	*Percent*	*Percent*
Control	3.62	8.31	2.91	0.98	0.15	6.44	4.1	77.5
−S	3.75	10.37	1.55	2.26	1.58	6.81	42.1	65.7
−N	4.33	8.97	3.42	3.73	.80	5.00	18.5	55.7
−P	3.33	10.30	1.96	3.02	.53	6.16	15.9	59.8
−Ca	2.68	4.72	.92	2.34	1.26	.83	47.0	17.6
−K	3.08	7.03	2.42	2.61	.39	3.88	12.7	55.2
−Fe	3.93	6.22	3.14	2.29	.28	2.96	7.1	47.8
−Mg	1.81	3.52	1.63	1.87	.06	.31	3.3	8.8

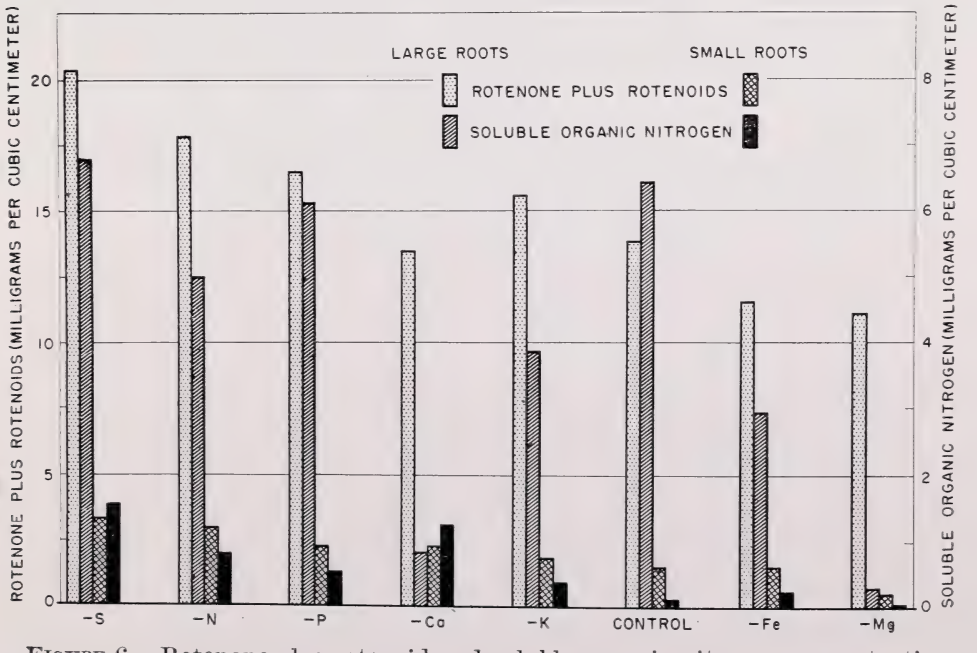

FIGURE 6.—Rotenone-plus-rotenoid and soluble organic nitrogen concentrations in large and small roots of control and mineral-deficient derris plants.

Differences in the physiological status of the plants when harvested influence the results. To illustrate, three of the control plants were undergoing rapid extension of new, thick, basal shoots and the fourth plant had just passed the crest of such a growth flush at that time. Under these conditions the concentration of soluble organic nitrogen would be temporarily high, particularly in large roots. Contrasting with the high value for soluble organic nitrogen in large control roots was the low value of this fraction in large −Ca roots. The depletion of starch from −Ca roots can be considered to have had a direct bearing on the soluble organic nitrogen value, inasmuch as carbohydrate reserves were apparently used to elaborate part of the soluble organic nitrogen (*6*, *33*, *34*). The distinct growth flushes described for −Ca plants were evidence that soluble organic nitrogen had probably accumulated at a higher concentration than was present at harvest.

Although the observations and data on −Mg plants were too limited to provide a completely satisfactory explanation of the extreme reduction in starch, it appears that factors other than the transformation of starch into the nitrogen-free components used in amide synthesis (*6*) were the main cause of starch depletion. Starch reserves, used in the production of feeble vine flushes, were not replaced because of the short life of the leaves. Also the efficiency of the photosynthetic processes might have been lowered by the restriction of magnesium (*21*).

The amide- and nitrate-nitrogen fractions were not correlated with root quality.

General Discussion of Growth Responses

Marked differences in growth occurred among the plants in the eight treatments. After having become well established, the control plants passed through successive cycles of rapid vegetative extension and relative dormancy. Frequently, the flushes of growth were confined to thick stems that grew from the base of the shoot system. The growth rhythm of −Fe and −N plants was essentially like that of the controls. In −S plants, the length of the cycle was noticeably protracted and stem elongation, when in progress, was remarkably rapid. The growth flushes of −Ca plants were generally not so clearly defined and were less vigorous than those of −S plants. Shoot extension in −Mg plants was slow and not characterized by distinct growth flushes: No vigorous shoots grew from the bases of stems, and dormancy eventually disappeared. Extreme stunting characterized both −P and −K plants: Even though the soluble organic nitrogen in these plants appeared to be ample for the onset of growth flushes, rapid vegetative extension did not occur.

That these variations in the responses of derris plants in the several treatments might have been caused by differences in auxin relations (*38*) is inferred by reports on other species of plants. Avery et al. (*3*) have shown that the accumulation of auxin in both horsechestnut and apple reached a peak just prior to the most rapid expansion of shoots, and that later the decline in auxin content and growth rate followed similar curves. Such a cycle of accumulation and depletion of auxin might be anticipated in all derris treatments with a definite rhythm of growth and relative dormancy. On the

other hand, dwarfism in corn (*37*) and the garden pea (*13*) have been correlated with auxin deficiency, and certain mineral deficiencies have been accompanied by a lowering of auxin concentration in several species of plants (*4*, *8*, *40*). By inference, the stunting of derris plants in the −K and −P treatments might have involved similar changes in the auxin mechanism.

Practical Applications of Results

Mild pathological symptoms, such as tipburn, brown spotting, and "white vein," that occur on the leaves of field-grown plants are not the result of the deficiency of any one of the elements studied. In all probability they are the responses of a forest liana to open-field conditions as suggested in the Introduction.

Provided that the proper class of nodule-forming bacteria are present and soil and climatic conditions are favorable (*29*), nitrogen in a chemical fertilizer has no special value. This conclusion, drawn from the data in tables 3 and 4, was supported by a field experiment with eight replications in which 100 pounds of nitrogen per acre applied as ammonium sulfate gave no significant increase in either the yield or quality of derris root (*28*).

The application of potash or phosphate fertilizers may increase the yield and quality of derris root by providing conditions favorable to rapid growth (*32*). That the minimal potassium requirements of *Derris* may be low is suggested by the results of Worsley (*47*), who reported that three plants, grown 18 months supposedly without potassium, yielded 215 grams of dry roots, as compared with 255 grams produced by controls. On the other hand, his three phosphorus-deficient plants yielded only 54 grams of dry roots. However, no critical study of the minimal potash and phosphate requirements of *Derris* has been reported; and it should be recalled that the fixation and availability of potassium (*7*, *16*) and phosphate (*39*) in soils have not been satisfactorily elucidated.

SUMMARY

1. A clone of *Derris elliptica* was grown for 1 year in sand cultures to study the results of deficiencies of S, N, P, Ca, K, Fe, and Mg.

2. The checking of shoot growth, common to all mineral deficiencies, was most severe in −K, −P, and −Ca treatments. Reduction in dry matter of roots was greatest in −K, −Ca, and −Mg treatments.

3. Specific patterns of chlorosis appeared in the leaves of all deficiencies except −N, −K, and −Ca.

4. When the ions withheld from the nutrient solutions were applied to young leaves, the characteristic symptoms of the mineral deficiencies did not develop in the treated tissues.

5. Dry roots of greenhouse plants supplied with a complete nutrient solution had 1.3 percent of rotenone, whereas dry roots of the same clone grown out-of-doors had 5.2 percent.

6. Root quality, expressed as the concentration of rotenone plus rotenoids, was highest in −S roots and lowest in −Mg roots.

7. The number, distribution, and staining properties of rotenoid cells were correlated with root quality.

8. Soluble organic nitrogen was directly correlated with root quality.

9. The literature indicates that alterations in the auxin mechanism might have influenced the growth responses of plants in several of the deficiency treatments.

10. The value of nitrogen in chemical fertilizers for *Derris* is questionable, but the application of potash and phosphate may increase both yield and quality of derris root.

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